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**14. ABSTRACT**

The funds utilized were to purchase a microscope to support several projects at Wright State University and at the Materials and Manufacturing Directorate at the Air Force Research Laboratory (AFRL/RX). The microscope purchased was the Leica DMI 6000B fully automated inverted microscope for phase and fluorescence with motorized stage with dry and oil immersion objectives, QImaging Retiga EXI camera with the RGB liquid crystal color filter slider module, Image Pro Plus 6.2 DMA software, shuttle computer with dual monitors to acquire and analyze the data. Three research projects that are in collaboration between WSU and AFRL/RX that will benefit from this microscope is Biomaterials Research, Biosensor Research and Cancer Biology.

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## Optical Inverted Microscope Imaging System for Biological and Non-Biological Samples

**DURIP Grant FA9550-07-1-0509**

**Final report – August 2008**

**Synoposis:** The optical inverted microscope imaging system purchased with the DURIP funding was the one described in the original proposal. Briefly, Leica DMI 6000B fully automated microscope with dry and oil immersion objectives was purchased along with the imaging software to acquire and analyze the data. Several training sessions have been setup by the company representative to train investigators using the microscope both at the Air Force Research laboratories (AFRL) as well as Wright State University (WSU). Additional training has been provided to AFRL personnel as needed.

**Instrument Description:** The microscope purchased was Leica DMI6000B fully automated inverted microscope (Figure 1) for phase and fluorescence with motorized stage, QImaging Retiga EXI camera with the RGB liquid crystal color filter slider module, Image pro Plus 6.2 MDA software, shuttle computer with dual monitors. A complete training was also included in the purchase and two training sessions were set up with representatives from the company and investigators from both AFRL and WSU. The three filters purchased included filters that detected DAPI stain and FITC and Texas red fluorochromes. In addition the objectives purchased included 10X, 20X and 40X dry immersion objectives and 63X and 100X oil immersion objectives.

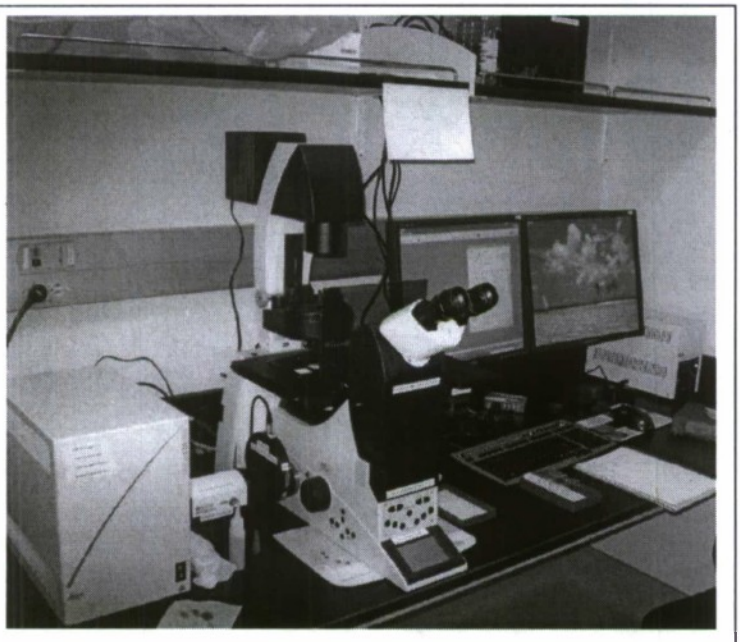

**Figure 1:** Leica DMI6000B Microscope in 125 Diggs Laboratory (Kadakia Lab) at Wright State University.

The microscope was purchased from W. Nuhsbaum, Inc. The information on the details of the microscope and the cost of the entire system was $76,068.00 with a detailed breakdown is shown in the in the Appendix. The microscope is currently located in the laboratory (125 Diggs Laboratory) of Dr. Kadakia on Wright state University campus.

**Projects Supported:** The acquisition has supported several projects at Wright State University and at the Materials and Manufacturing Directorate at the Air Force Research Laboratory (AFRL/RX).

*Biomaterials Research:* This is a joint collaboration between my group and the biotechnology group at AFRL/RX. In the project we are developing silk as a biomaterial for cell scaffold. The microscope has enabled us to obtain some high quality images of mammalian cells growing on patterned silk films. This work was published in Langmuir. Currently, the microscope is been used to observed the binding of phage displaying specific peptides to various fibers (silk and cotton). Phage peptides that have an affinity for silk or cotton fibers were obtained using a phage display library. The microscope allows the researchers to determine the binding specificity of the phage peptide clones to the various fibers using immunofluorescence microscopy.

*Biosensor Research:* Researchers from AFRL/RX have been using the microscope to observe the binding of fluorescently labeled analogue of TNT to yeast cell surfaces. The yeast cell surface was engineered to display an engineered insect odor binding protein (Asp2). The Asp2 protein is believed to bind TNT based on previous work done by the AFRL/RX group. The microscope enabled the researchers to observe the binding of the fluorescently labeled analogue to the yeast cell surface using the fluorescence capability of the microscope. In addition, Brightfield imaging was also done to observe the yeast cells.

*Cancer Biology:* Researchers at Wright State University, including the Kadakia group, has been using the

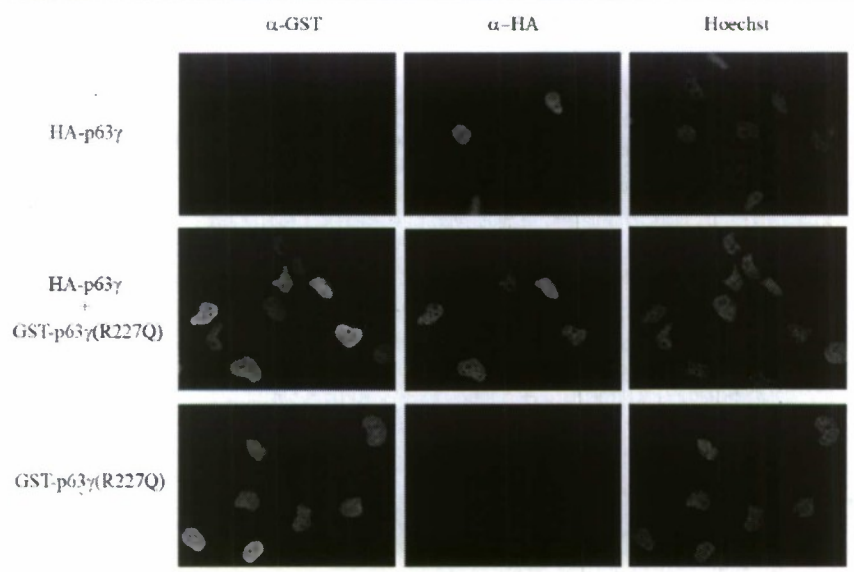


Figure 2: TAp63γ mutants do not affect the localization of wildtype TAp63γ. H1299 cells were transfected with HA-tagged TAp63γ alone or along with GST tagged TAp63γ mutants as indicated. At 24 hr post transfection immunofluorescence assay was performed. Wild type TAp63γ and TAp63γ mutant expression was detected using mouse anti-HA and rabbit anti-GST primary antibodies respectively and subsequently with corresponding fluorescently-tagged secondary antibodies. The nucleus was stained with Hoechst dye and the cells were examined using fluorescence microscope.

microscope at its various capabilities for immunofluorescence staining of cells that express various transfected genes like p63, p73 and other cancer markers. As shown in Figure 2, H1299 cells were plated on sterilized coverslips and at 24 hr after seeding, expression plasmids encoding HA-tagged wildtype TAp63γ or GST tagged TAp63γ mutants were transiently transfected either alone or in combination. Primary antibodies used to detect HA-TAp63γ and GST tagged mutants were mouse monoclonal anti-HA 12CA5 (Roche Diagnostics, Indianapolis, IN) at a dilution of 1:100 and rabbit polyclonal anti-GST Z5 (Santa Cruz Biotechnology, Santa Cruz, CA) at 1:200. Fluorescently-labeled secondary goat anti-rabbit, fluorescein isothiocyanate (FITC)-conjugated immunoglobulin G (IgG) antibody (Jackson Immunoresearch, West Grove, PA, USA) and secondary donkey anti-mouse, Texas red dye-conjugated IgG antibody (Jackson Immunoresearch, West Grove, PA, USA) were used. Hoechst dye 33342 (Sigma, St. Louis, MO) was used for nuclear staining. Preparations were examined using the Leica 6000 Microscope.

Publications:

1. Gupta, M. K., Khokhar, S. K., Phillips, D. M., Caserta, T. M., Sowards, L. A. Drummy, L. F., Kadakia, M. P. & Naik, R. R. (2007) Patterned Silk Films Cast from Ionic Liquid Solubilized Fibroin as Scaffolds for Cell Growth. Langmuir 23, 1315-1319.

2. Khokhar, S. K., Kommagani, R., & Kadakia, M. P. (2008) Differential Effects of p63 Mutants on Transactivation of p53 and/or p63 responsive genes. Cell Research 1-13.